

Testing the uniqueness of *Z. h. intermedius* relative to *Z. h. campestris*

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Background

This report is a supplement to Ramey et al. (2004) and incorporates new data and analyses using samples of *Zapus hudsonius* obtained from the range of *Z. h. intermedius*. The US Fish and Wildlife Service requested this additional information and provided funding under a cooperative agreement. This additional information is relevant to the question of whether the population of what is now called *Z. h. preblei* could be considered a distinct population segment (DPS) under the Endangered Species Act (ESA). It is also relevant to the question of whether *Z. h. campestris* (including *Z. h. preblei*) could be considered for endangered or threatened listing. If *Z. h. campestris* (including *Z. h. preblei*) is to be considered for listing under the ESA, it is necessary to first determine whether this subspecies actually inhabits a broader geographic area than is reflected in the current taxonomy.

Methods

Morphometric analyses

To test the multivariate morphometric distinguishability of *Z. h. intermedius* from *Z. h. campestris*, we used linear discriminant analysis. Based on the results of previous analyses (Ramey et al. 2004), we combined the *Z. h. preblei* specimens with those of *Z. h. campestris* and compared this combined sample to a sample of 37 *Z. h. intermedius* specimens. Methods were the same as those described in (Ramey et al. 2004).

Population genetic and phylogenetic analyses

We used methods described previously (Ramey et al. 2004) and include 47 additional mtDNA control region sequences obtained from *Z. h. intermedius* specimens (Table 1, Figure 1). Tajima's *D* was used as a test of selective neutrality for mtDNA, using ARLEQUIN 2.0.

MDIV

Fixation indices such as F_{ST} (Wright 1921) are calculated under assumptions of equilibrium; any shared genetic variation is therefore assumed to be the result of current gene flow. Thus F_{ST} cannot distinguish between recently-isolated populations with no gene flow and populations isolated for a longer period of time but with continuing low levels of gene flow. As an alternative, we used the maximum-likelihood based program MDIV (Nielsen & Wakeley 2001) to evaluate whether shared mtDNA variation between *Z. h. preblei*, *Z. h. campestris*, *Z. h. intermedius*, *Z. h. pallidus*, and *Z. h. luteus* reflected very recent (including current) gene flow or complete, but recent, isolation.

MDIV uses Markov-chain Monte Carlo simulations to estimate for two populations the likelihood of the parameters *theta* ($4N_e\mu$) and *M* ($2N_e m$), where N_e is the effective population size, *m* is the migration rate, and μ is the mutation rate. MDIV assumes that N_e and *m* are the same for both populations. We used MDIV to estimate migration (*m*) between *Z. h. preblei* and *Z. h. campestris*, and to compare this estimate of gene flow to estimates of gene flow between other pairs of populations. We ran

5,000,000 chains for each simulation with burn-in of 500,000 chains, set T_{MAX} and M_{MAX} to 10, and used the HKY model of sequence evolution. Parallel simulations gave similar results suggesting that this number of chains was adequate. MDIV tests a wide range of values for each parameter and calculates the likelihood of each tested value. We calculated confidence intervals around the parameter estimates using Akaike's Information Criterion (AIC) (Burnham & Anderson 1998) to determine the range of parameter values that were not significantly less likely than the best estimated value (Nielsen & Wakeley 2001). Because the number of parameters was fixed, we calculated AIC as:

$$AIC = -2 * \log(\text{likelihood})$$

We accepted parameter values within 2 AIC units on either side of the most likely estimated parameter value. However, due to the assumptions made by MDIV about N_e and m , these confidence intervals may be understated. We calculated N_e from the estimate of theta using $\mu = 2.5 * 10^{-5}$ over 346 base pairs (estimated from divergence in vole mtDNA control region sequences by Matson and Baker (2001)). We converted M to m using the most likely estimate of N_e .

Results

Discriminant analysis

Four variables were determined to have the greatest discriminating power using forward and backward stepwise procedures. These included condylobasal length, zygomatic length, mastoidal length, and interorbital breadth. A combined sample of *Z. h. preblei* and *Z. h. campestris* ($n=71$) were used in the discriminant analysis comparisons with *Z. h. intermedius*. Discriminating ability with a jackknifed posterior probability of ≥ 0.95 was poor, with 46% (48 of 105) of the specimens correctly classified to each of the two population considered (*Z. h. intermedius* and the pooled *Z. h. preblei/Z. h. campestris*). (Note: incomplete specimens could not be used in discriminant analysis.) In comparison, Conner and Shenk (2002) found a high degree of classification certainty between **species** of jumping mice in Colorado and Wyoming with $>96\%$ of specimens correctly classified at a posterior probability >0.95 .

mtDNA summary statistics

Sixteen haplotypes were identified in *Z. h. intermedius* (Table 1) and nucleotide diversity was 0.012 (SD=0.00678).

mtDNA test for selective neutrality

Values of Tajima's D were not significant ($p>0.05$) for subspecies considered individually or pooled together. Therefore, the null hypothesis of selective neutrality for mtDNA could not be rejected.

mtDNA AMOVA

Greater variation was found within (69.3%) than among the subspecies (30.7%) *Z. h. intermedius*, *Z. h. campestris*, and *Z. h. preblei*. When only *Z. h. intermedius*, and *Z. h. campestris* were compared, greater variation was found within *Z. h. intermedius*, and *Z. h. campestris* (96.2%) than among those two subspecies (3.8%). When *Z. h. campestris*,

and *Z. h. preblei* were considered as a single subspecies and compared with *Z. h. intermedius*, the following results were obtained: 81.54% of the variation was found within subspecies and 18.46% of the variation was found between subspecies. Based on these analyses, *Z. h. intermedius* fails our test of genetic uniqueness for mtDNA relative to *Z. h. campestris*.

mtDNA MDIV

After correcting for N_e , *Z. h. preblei* and *Z. h. campestris* showed low, but non-zero, levels of very recent gene flow (m and M) (Table 2). Thus, the null hypothesis of no very recent gene flow between these putative subspecies can be rejected. Gene flow between *Z. h. campestris* and *Z. h. intermedius* was also non-zero, therefore the null hypothesis of no very recent gene flow can also be rejected for these putative subspecies. In comparison, the range of migration estimates between *Z. h. preblei* - *Z. h. luteus*, *Z. h. preblei* - *Z. h. pallidus*, and *Z. h. preblei* - *Z. h. intermedius* included zero, indicating that little or no very recent gene flow occurs between *Z. h. preblei* and those subspecies.

Phylogenetic analyses

All phylogenetic analyses (maximum parsimony, maximum likelihood, and neighbor-joining) resolved a two strongly supported *Z. hudsonius* clades (>85% bootstrap support). These included a *Z. h. preblei/Z. h. campestris/Z. h. intermedius* clade and a *Z. h. luteus/Z. h. pallidus* clade.

Nearly all of the *Z. h. intermedius* haplotypes (except one) were found in the *Z. h. preblei/Z. h. campestris/Z. h. intermedius* clade. Four of the *Z. h. intermedius* haplotypes were identical to those found in *Z. h. campestris* (Table 1, Figure 2). One *Z. h. intermedius* haplotype clustered with individuals on the *Z. h. luteus/Z. h. pallidus* clade.

Discussion

Our morphometric results failed to reliably distinguish *Z. h. intermedius* from a combined sample of *Z. h. campestris* and *Z. h. preblei*. Ability to distinguish them morphometrically was no better than random flips of a coin.

Our mtDNA results are consistent with our morphometric findings. The mtDNA sequences examined appear to be selectively neutral and therefore useful for population genetic and phylogenetic analyses. AMOVA results reveal that greater variance is found within than among *Z. h. preblei*, *Z. h. campestris*, and *Z. h. intermedius*, suggesting a lack of strong genetic structure between these putative subspecies.

MDIV estimates of migration between contiguous populations suggested that *Z. h. preblei* is linked to *Z. h. campestris*, and that *Z. h. campestris* is linked to *Z. h. intermedius* by very recent gene flow. While the simplifying assumptions made by MDIV preclude exact interpretation of estimates of N_e , m , or M , these populations are better linked by very recent gene flow than the other population comparisons considered. *Z. h. preblei* does not appear to be linked by very recent flow to *Z. h. intermedius*, however, this result is consistent with a stepping stone model of migration (Figure 1). The

estimated migration rate (m) between *Z. h. preblei* and *Z. h. campestris* was greater than that estimated between other contiguous populations including *Z. h. campestris* - *Z. h. intermedius* and *Z. h. pallidus* - *Z. h. intermedius* (Table 2). We found no clear evidence of very recent migration between *Z. h. preblei* and the geographically-discontinuous *Z. h. luteus* nor with *Z. h. pallidus* to the east of the *Z. h. preblei* population. The few haplotypes and low nucleotide diversity found in *Z. h. preblei* are consistent with a founder event and relatively recent colonization of this area.

Our morphometric and mtDNA results are consistent with those of Jones (1981) and suggest that *Z. h. preblei*, *Z. h. campestris*, and *Z. h. intermedius* appear be a single subspecies. If these were synonymized, they would be named the Prairie jumping mouse (*Z. h. campestris*) (Preble 1899).

Literature Cited

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Table 1. Specimens of *Z. hudsonius* used in phylogenetic and population genetic analyses. These are listed by museum or tissue archive catalog number (DMNH = Denver Museum of Nature & Science; TK = Texas Tech; KU = University of Kansas; UNSM = University of Nebraska State Museum; MSB and NK = Museum of Southwestern Biology; PIONEER = Pioneer Environmental Services.). Abbreviations for states are as follows: AZ = Arizona; CO = Colorado; IL = Illinois; IN = Indiana; IA = Iowa; KS = Kansas; MO = Missouri; MT = Montana; NM = New Mexico; NE = Nebraska; SD = South Dakota; WY = Wyoming. State abbreviations are followed by counties. The location of haplotypes in the table approximately corresponds to the location of the haplotypes in the neighbor-joining tree. The *Z. h. intermedius* specimens are highlighted in bold.

Representative individuals used in phylogenetic analysis	Additional specimens with identical mtDNA haplotype: ID, state, and county	subspecies	haplotype
MSB40951, AZ:Apache	MSB40994, AZ:Apache	Z.h. luteus Z.h. luteus	L6
MSB89194, AZ:Navajo		Z.h. luteus	L5
MSB86244, AZ:Apache	MSB91627, AZ:Navajo MSB91675, AZ:Apache NK1584, AZ:Apache DMNH8635, CO:Las Animas DMNH8633, CO:Las Animas KU41451, WY:Crook KU153706, KS:Leavenworth KU112661, SD: Lawrence	Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. campestris Z.h. pallidus Z.h. campestris	L/PAL/C2
UNSM20596, NE:Buffalo		Z.h. pallidus	PAL10
UNSM26492, NE:Buffalo	UNSM20879, NE:Buffalo	Z.h. pallidus Z.h. pallidus	PAL9
UNSM13217, NE:Cherry	UNSM12980, NE:Garden UNSM12991, NE:Garden UNSM26316, NE:Hall UNSM20744, NE:Hall UNSM20747, NE:Hall UNSM26462, NE:Merrick UNSM13067, NE:Thomas	Z.h. pallidus Z.h. pallidus Z.h. pallidus Z.h. pallidus Z.h. pallidus Z.h. pallidus Z.h. pallidus Z.h. pallidus	PAL8
KU116269		Z.h. intermedius	INT-XVI
UNSM17482, NE:Antelope	UNSM17495, NE:Antelope UNSM17498, NE:Antelope UNSM17499, NE:Antelope UNSM13084, NE:Dixon UNSM14008, NE:Dodge UNSM13118, NE:Holt UNSM13343, NE:Lancaster	Z.h. pallidus Z.h. pallidus Z.h. pallidus Z.h. pallidus Z.h. pallidus Z.h. pallidus Z.h. pallidus Z.h. pallidus	PAL7
UNSM13119, NE:Holt	UNSM13065, NE:Thomas	Z.h. pallidus Z.h. pallidus	PAL6
UNSM17727, NE:Boyd		Z.h. pallidus	PAL5
UNSM20600, NE:Buffalo	KU109633, KS:Osage KU109634, KS:Osage	Z.h. pallidus Z.h. pallidus Z.h. pallidus	PAL4

KU153597, MO:Macon	KU153598, MO:Macon KU153784, KS:Douglas KU153707, KS:Leavenworth	Z.h. pallidus Z.h. pallidus Z.h. pallidus Z.h. pallidus	PAL3
MSB37154, NM:Otero	MSB61696, NM:Otero MSB61684, NM:Otero MSB61690, NM:Otero MSB61693, NM:Otero MSB61712, NM:Otero MSB58369, NM:Rio Arriba NK871, NM:Otero NK884, NM: Socorro DMNH8630: CO:Las Animas	Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus	L4
DMNH8631, CO:Las Animas		Z.h. luteus	L3
DMNH8632, CO:Las Animas	DMNH8634, CO:Las Animas	Z.h. luteus Z.h. luteus	L2
NK9976, NM:Bernalillo		Z.h. luteus	L1
MSB58370, NM:Rio Arriba	MSB56980, NM:Sandoval MSB56986, NM:Sandoval MSB56987, NM:Sandoval MSB56991, NM:Sandoval MSB56993, NM:Sandoval MSB62096, NM:Sandoval MSB62103, NM:Valencia NK856, NM:Sandavol KU112665, SD:Lawrence KU109963, SD:Lawrence KU110033, SD:Bennett	Z.h. luteus Z.h. campestris Z.h. campestris Z.h. pallidus	L/PAL/C1
KU110022, SD:Bennett		Z.h. pallidus	PAL2
UNSM27388, SD:Clay	UNSM27389, SD:Clay KU116266 , IO:Buena Vista KU140721, SD:Brown KU153190 , SD:Walworth KU153209 , SD:Minnehaha KU153212 , SD:Minnehaha KU153221 , SD:Moody	Z.h. pallidus Z.h. pallidus Z. h. intermedius Z. h. intermedius Z. h. intermedius Z. h. intermedius Z. h. intermedius Z. h. intermedius	PAL1/ INT-XV
KU147020 , SD:Brown	KU153176 , SD:Brown KU153177 , SD:Brown KU153180 , SD:Brown KU153181 , SD:Brown	Z. h. intermedius Z. h. intermedius Z. h. intermedius Z. h. intermedius Z. h. intermedius	INT-XIV
KU101564, SD:Pennington	DMNH10638/TK86190, WY:Weston DMNH10639/TK86191, WY:Weston KU101558, SD:Pennington KU123593, MT:Carter KU123598, MT:Carter KU123599, MT:Carter KU115700, ND:Burleigh KU115702, ND:Burleigh KU115710, ND:Burleigh	Z.h. campestris Z.h. campestris Z.h. campestris Z.h. campestris Z.h. campestris Z.h. campestris Z.h. campestris Z. h. intermedius Z. h. intermedius Z. h. intermedius	C8/10/ INT-VI

	KU115731 , SD:Walworth KU115732 , SD:Walworth KU120018 , ND:Burleigh KU120019 , ND:Burleigh KU123021 , ND:Dunn KU123022 , ND:Dunn KU123031 , ND:Dunn KU123032 , ND:Dunn KU159190 , SD:Walworth DMNS7764 , ND: Mercer	Z. h. intermedius Z. h. intermedius	
KU123033 , ND:Dunn		Z.h. intermedius	INT-I
KU112663, SD:Lawrence	KU115730 , SD:Walworth	Z.h. campestris Z. h. intermedius	C9/INT-VII
KU20839, WY:Crook		Z.h. campestris	C7
KU83559, SD:Harding		Z.h. campestris	C6
KU20844, WY:Crook	KU42471, WY:Weston KU87040, SD:Harding KU83557, SD:Harding KU87042, SD:Harding KU112660, SD:Lawrence KU115895 , SD:Harding KU115896 , SD:Harding KU115897 , SD:Harding	Z.h. campestris Z.h. campestris Z.h. campestris Z.h. campestris Z.h. campestris Z.h. campestris Z. h. intermedius Z. h. intermedius Z. h. intermedius	C5/INT-XIII
KU20843, WY:Crook		Z.h. campestris	C4
KU109970, SD:Lawrence		Z.h. campestris	C3
KU120017 , ND:Burleigh		Z. h. intermedius	INT-IX
KU42469, WY:Weston		Z.h. campestris	C2
KU101552, SD:Pennington		Z.h. campestris	C1
KU116263 , IO:Emmet	KU116265 , IO:Plymouth KU147018 , SD:Deuel KU153196 , SD:Deuel KU153203 , SD:Lincon		INT-XII
KU153201 , SD:Deuel			INT-V
DMNH10614/TK86183, CO:El Paso	DMNH10331/TK86088, CO:Teller DMNH10606/TK86165, CO:El Paso DMNH10604/TK86169, CO:El Paso DMNH10612/TK86170, CO:El Paso DMNH10605/TK86173, CO:El Paso DMNH10618/TK86182, CO:El Paso DMNH10611/TK86185, CO:El Paso DMNH10635/TK86196, CO:Douglas KU109972, SD:Custer	Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. campestris	C/P4
DMNH9204/XM871, CO:Boulder	DMNH9205/XM872, CO:Boulder DMNH9312/XM874, CO:Gilpin DMNH9046/XM876, CO:Boulder DMNH9314/XM877, CO:Boulder DMNH9203/TK51406, CO:Jefferson DMNH9880/TK86021, CO:Boulder DMNH9854/TK86026, CO:Douglas DMNH9876/TK86029, CO:Douglas	Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii	C/P3

	DMNH9857/TK86030, CO:Douglas DMNH9865/TK86031, CO:Douglas DMNH9868/TK86032, CO:Douglas DMNH9843/TK86034, CO:Boulder DMNH10169/TK86048, CO:Boulder DMNH10266/TK86080, CO:Douglas DMNH10269/TK86083, CO:Douglas DMNH10354/TK86090, CO:Boulder DMNH10408/TK86098, WY:Albany DMNH9564/TK86105, CO:Boulder DMNH9561/TK86109, CO:Larimer DMNH9576/TK86115, CO:Douglas DMNH9574/TK86116, CO:Douglas DMNH10520/TK86124, CO:Jefferson DMNH10602/TK86163, CO:Elbert KU110013, SD:Custer KU123597, MT:Carter	Z.h. prebleii Z.h. campestris Z.h. campestris	
DMNH9579/XM1166, CO:El Paso	DMNH9313/XM875, CO:El Paso DMNH9315/XM879, CO:El Paso DMNH10380/TK86093, CO:El Paso DMNH9565/TK86106, CO:El Paso DMNH9563/TK86107, CO:El Paso DMNH9566/TK86118, CO:El Paso DMNH9573/TK86120, CO:Douglas DMNH9572/TK86121, CO:Douglas DMNH9571/TK86122, CO:Douglas DMNH9574/TK86166, CO:El Paso DMNH10607/TK86167, CO:El Paso KU109978, SD:Custer KU123592, MT:Carter	Z.h. prebleii Z.h. campestris Z.h. campestris	C/P2
DMNH10405/TK86095, WY:Albany	DMNH10258/TK86074, WY:Laramie DMNH10270/TK86081, CO:Larimer DMNH10404/TK86094, WY:Platte DMNH10406/TK86096, WY:Albany DMNH10407/TK86097, WY:Albany DMNH9568/TK86117, CO:Larimer PIONEER9A43, CO: Larimer PIONEER9B89, CO:Larimer KU109984, SD:Custer KU109985, SD:Custer	Z.h. prebleii Z.h. campestris Z.h. campestris	C/P1
KU104062 , IO:Winneshiek	KU116264 , IO:Emmet KU153229 , SD:Union KU153203 , SD:Lincon	Z.h. intermedius Z.h. intermedius Z.h. intermedius Z.h. intermedius	INT-VIII
KU140722 , SD:Brown		Z.h. intermedius	INT-X
KU153205 , SD:Lincon	KU153215 , SD:Minnehaha	Z.h. intermedius Z.h. intermedius	INT-XI
KU127252 , IL:Henry		Z.h. intermedius	INT-IV
KU112830 , IN:Wayne		Z.h. intermedius	INT-III
KU108068 , IA:Marion		Z.h. intermedius	INT-II

Table 2. Maximum likelihood (MDIV) estimates of very recent gene flow between populations of *Zapus hudsonicus*, where $\theta = 4N_e\mu$, N_e is the estimated effective population size, m is migration rate between populations, and M is the scaled migration rate. Range of m defined as within 2 AIC units of most likely parameter value.

Comparison	θ	N_e	m (range)	M (range)
<i>Z. h. preblei</i> - <i>Z. h. campestris</i>	2.7	27,409	$3.3 \times 10^{-6} - 3.2 \times 10^{-5}$	0.18 - 1.74
<i>Z. h. campestris</i> - <i>Z. h. intermedius</i>	23.0	230,924	$1.3 \times 10^{-6} \times 1.3 \times 10^{-5}$	0.58 - 5.86
<i>Z. h. pallidus</i> - <i>Z. h. intermedius</i>	10.5	105,622	$1.9 \times 10^{-7} - 2.3 \times 10^{-6}$	0.04 - 0.48
<i>Z. h. preblei</i> - <i>Z. h. luteus</i>	5.6	56,124	$0 - 1.0 \times 10^{-6}$	0.0 - 0.14
<i>Z. h. preblei</i> - <i>Z. h. pallidus</i>	6.4	64,558	$0 - 2.2 \times 10^{-6}$	0.0 - 0.28
<i>Z. h. preblei</i> - <i>Z. h. intermedius</i>	19.1	191,767	$0 - 2.2 \times 10^{-6}$	0.0 - 0.84

Figure 1. Map showing collection locations of specimens used in mtDNA analyses. Subspecies ranges are indicated. Pink circles indicate specimens on the *Z. h. preblei*/*Z. h. campestris*/*Z. h. intermedius* clade, green squares indicate specimens on the *Z. h. luteus*/*Z. h. pallidus* clade. MtDNA and morphometric results suggest synonymy for both *Z. h. preblei* and *Z. h. intermedius* with *Z. h. campestris*.

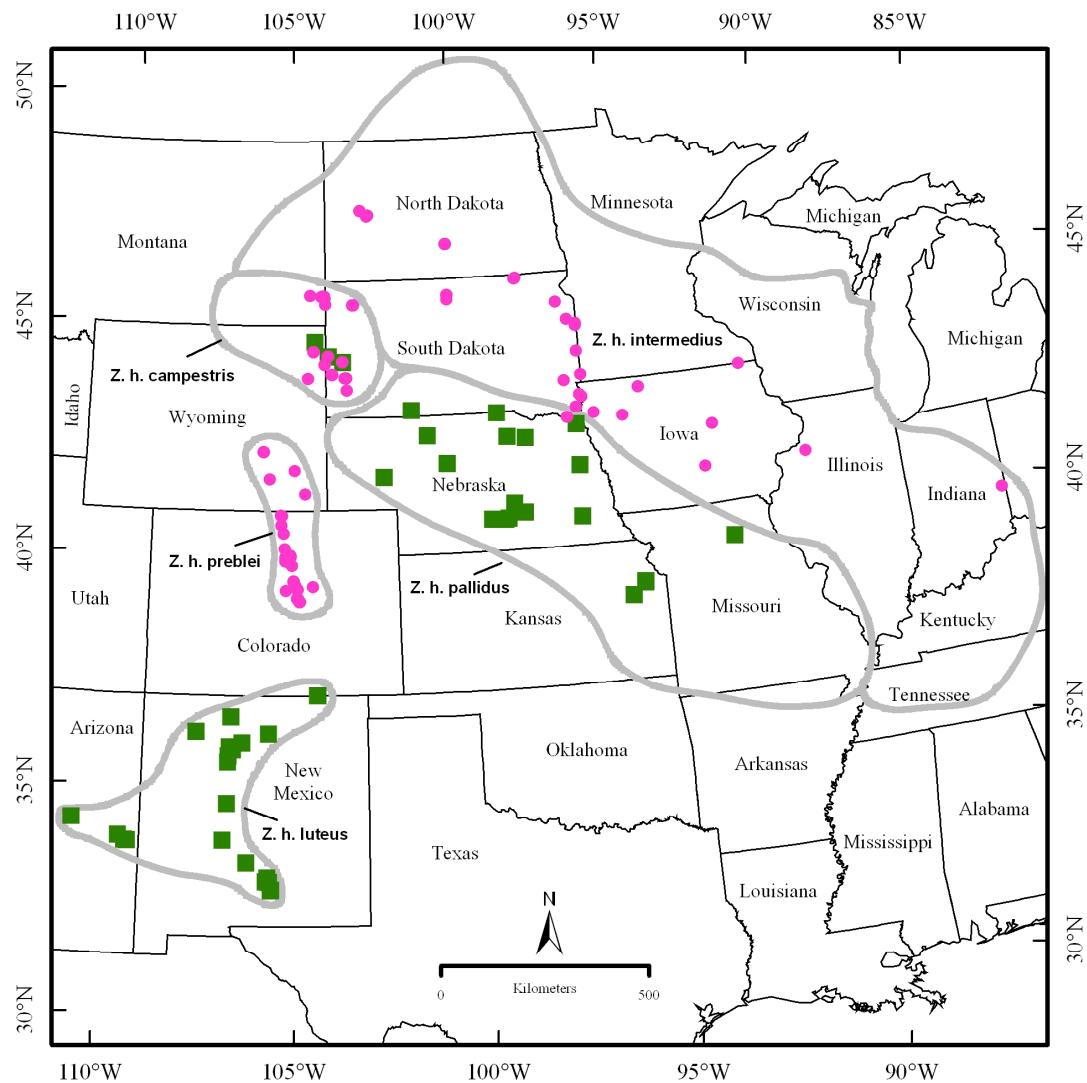


Figure 2. Neighbor joining tree of mtDNA sequences used in this study. All phylogenetic analyses (maximum parsimony, maximum likelihood, and neighbor-joining) resolved two strongly supported *Z. hudsonius* clades (>85% bootstrap support). These included a *Z. h. preblei/Z. h. campestris/Z. h. intermedius* clade and a *Z. h. luteus/Z. h. pallidus* clade. Colors indicate subspecies: *Z. h. preblei* (blue), *Z. h. campestris* (pink), *Z. h. intermedius* (red), *Z. h. luteus* (dark green), and *Z. h. pallidus* (light green).

